Application of CLSM and 3-D reconstruction techniques to the localization of allergenic proteins in olive (*Olea europaea* L.) pollen

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Several olive pollen allergens have been identified and characterized at the molecular level. The effects of these allergenic proteins in humans are well defined, but not their role and function in the pollen grain. Cellular localization of allergenic proteins allows us to obtain important clues regarding these biological functions, such as their place of synthesis and storage, the presence of post-translational modifications, the mechanisms involved in allergen release from the pollen grain once it becomes in contact with physiological fluids, and the interaction of allergens with ligands, substrates and co-factors.

Diverse microscopy techniques have been used (videomicroscopy, confocal laser scanning microscopy, deconvolution and image improvement, colocalization and three-dimensional reconstruction techniques) to analyze allergens localization throughout pollen development and in vitro pollen germination.

Most allergens studied (Ole e 1, Ole e 2, Ole e 9 and Ole e 10) have been localized by using monoclonal and polyclonal antibodies in immunocytochemical experiments. These experiments have been used in combination with the detection of substrates like callose by using the sirofluor fluorochrome, and the autofluorescence of the pollen wall. Image capture was performed in a Nikon C1 confocal microscope, equipped with a 3-laser unit and a transmitted light channel.

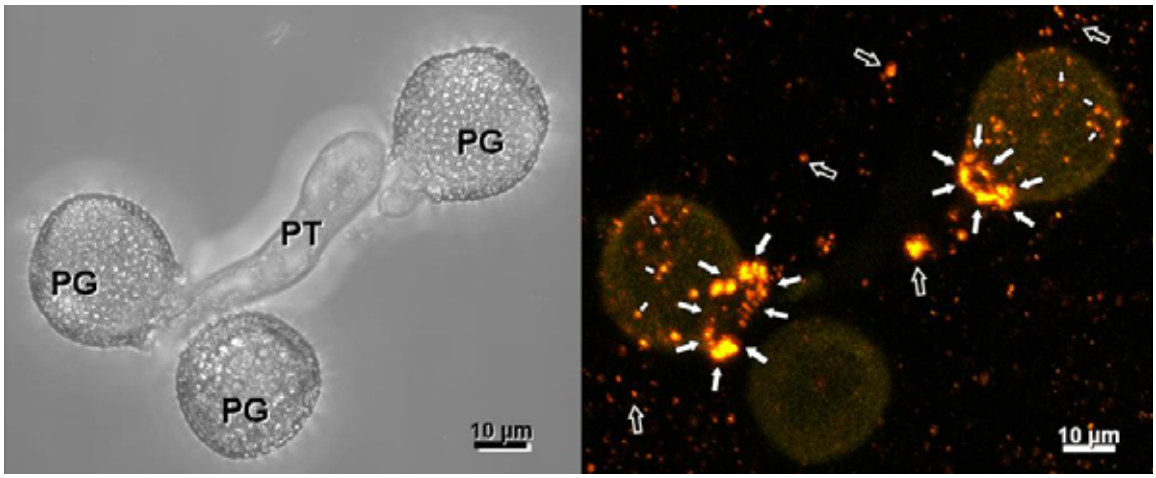

Figure 1: Immunolocalization of profilin allergen (ole e 2) in olive pollen grains germinating *in vitro* by using a Cy3 secondary labelled antibody. The allergen is localized in the aperture regions (large arrows), the pollen wall (small arrows) and the germination medium (empty arrows). Left panel: transmitted light channel. Right panel: Projection after 3-D reconstruction of 30 images. PG: pollen grain. PT: pollen tube.

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